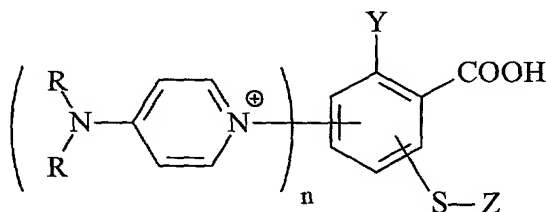


We claim:

1. A rhodamine dye or a salt thereof, comprising a rhodamine-type parent xanthene ring having attached to the xanthene C9 carbon a phenyl group that is further substituted with an ortho carboxy or ortho sulfonate group or a salt thereof, one to three substituted or unsubstituted aminopyridinium groups and a substituted or unsubstituted alkylthio, arylthio or heteroarylthio group, said rhodamine dye optionally including one or more linking moieties.

2. The rhodamine dye of Claim 1 which comprises the structure:



wherein:

n is 1, 2, or 3;

Y is a rhodamine-type parent xanthene ring attached to the illustrated phenyl group at the xanthene C9 carbon;

each R is independently selected from the group consisting of (C<sub>1</sub>-C<sub>6</sub>) alkyl and heteroalkyl, (C<sub>5</sub>-C<sub>20</sub>) aryl and heteroaryl, (C<sub>6</sub>-C<sub>26</sub>) arylalkyl and heteroalkyl, (C<sub>5</sub>-C<sub>20</sub>) arylaryl and heteroaryl-heteroaryl, or when taken together, R is (C<sub>4</sub>-C<sub>10</sub>) alkylidiyl, (C<sub>4</sub>-C<sub>10</sub>) alkyleno,

heteroalkylidiyl and heteroalkyleno;

S is sulfur;

Z is (C<sub>1</sub>-C<sub>12</sub>) alkyl, (C<sub>1</sub>-C<sub>12</sub>) alkyl substituted with one or more of the same or different W<sup>1</sup> groups, (C<sub>5</sub>-C<sub>20</sub>) aryl and heteroaryl, and (C<sub>5</sub>-C<sub>20</sub>) aryl and heteroaryl substituted with one or more of the same or different W<sup>2</sup> groups;

W<sup>1</sup> is selected from the group consisting of -X, -R, =O, -OR, -SR, =S, -NRR, =NR, -CX<sub>3</sub>, -CN, -OCN, -SCN, -NCO, -NCS, -NO, -NO<sub>2</sub>, =N<sub>2</sub>, -N<sub>3</sub>, -S(O)<sub>2</sub>O<sup>-</sup>, -S(O)<sub>2</sub>OH, -S(O)<sub>2</sub>R, -C(O)R, -C(O)X, -C(S)R, -C(S)X, -C(O)OR, -C(O)O<sup>-</sup>, -C(S)OR, -C(O)SR, -C(S)SR, -C(O)NRR, -C(S)NRR and -C(NR)NRR;

W<sup>2</sup> is selected from the group consisting of -R, -OR, -SR, -NRR, -S(O)<sub>2</sub>O<sup>-</sup>,

$-\text{S}(\text{O})_2\text{OH}$ ,  $-\text{S}(\text{O})_2\text{R}$ ,  $-\text{C}(\text{O})\text{R}$ ,  $-\text{C}(\text{O})\text{X}$ ,  $-\text{C}(\text{S})\text{R}$ ,  $-\text{C}(\text{S})\text{X}$ ,  $-\text{C}(\text{O})\text{OR}$ ,  $-\text{C}(\text{O})\text{O}^-$ ,  $-\text{C}(\text{S})\text{OR}$ ,  
 $-\text{C}(\text{O})\text{SR}$ ,  $-\text{C}(\text{S})\text{SR}$ ,  $-\text{C}(\text{O})\text{NRR}$ ,  $-\text{C}(\text{S})\text{NRR}$  and  $-\text{C}(\text{NR})\text{NRR}$ ;

each X is independently a halogen; and

Y or Z is optionally substituted with L where L is a bond or a linker.

5

3. The rhodamine dye of Claim 2 in which L is selected from a hydrophobic moiety, a charged group, a member of a pair of specific binding molecules, a photo-activatable group and a reactive functional group.

10

4. The rhodamine dye of Claim 2 where Z has the form  $\text{Z}^1\text{-L-R}_x$ , or a salt thereof, wherein:

$\text{Z}^1$  is  $(\text{C}_1\text{-C}_{12})$  alkylidyl,  $(\text{C}_1\text{-C}_{12})$  alkylidyl independently substituted with one or more of the same or different  $\text{W}^1$  groups, or

$(\text{C}_5\text{-C}_{14})$  arylidyl, and arylidyl, heteroarylidyl and heteroarylidyl independently substituted

15

with one or more of the same or different  $\text{W}^2$  groups;

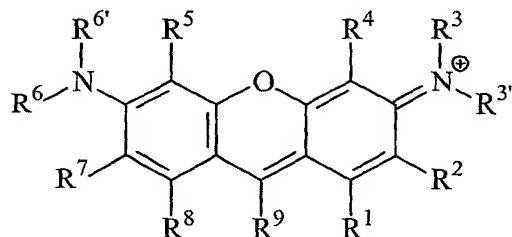
L is a bond or a linker; and

$\text{R}_x$  is a reactive functional group.

5. The rhodamine dye of Claim 4 in which Y is selected from:

20

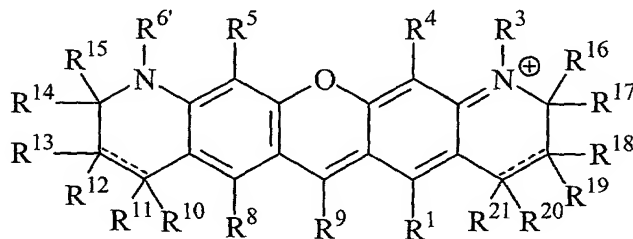
(Y-1)



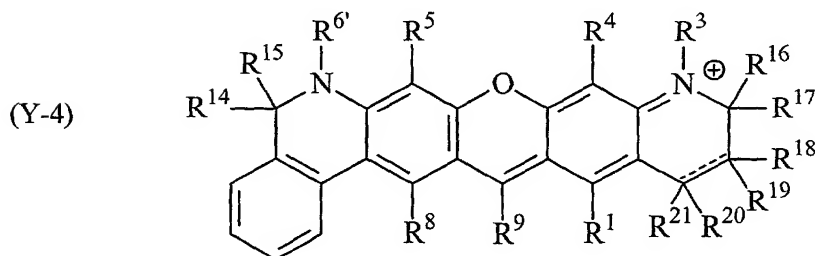
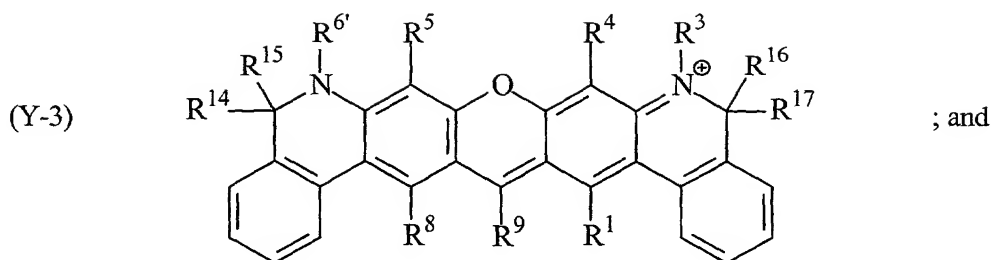
;

25

(Y-2)



;



and a salt thereof, wherein:

$R^1$  and  $R^2$  when taken alone, are independently hydrogen or  $(C_1-C_6)$  alkyl;

10  $R^3$  and  $R^3'$  when taken alone, are independently selected from the group consisting of hydrogen,  $(C_1-C_6)$  alkyl,  $(C_5-C_{14})$  aryl and arylaryl, or when taken together is  $(C_4-C_6)$  alkylidyl or alkyleno, or when individually taken together with  $R^2$  or  $R^4$  is  $(C_2-C_6)$  alkylidyl or  $(C_2-C_6)$  alkyleno;

$R^4$ , when taken alone, is selected from the group consisting of hydrogen and  $(C_1-C_6)$  alkyl, or when taken together with  $R^3$  or  $R^3'$  is  $(C_2-C_6)$  alkylidyl or alkyleno;

15  $R^5$ , when taken alone, is selected from the group consisting of hydrogen and  $(C_1-C_6)$  alkyl, or when taken together with  $R^6$  or  $R^6'$  is  $(C_2-C_6)$  alkylidyl or alkyleno;

$R^6$  and  $R^6'$  when taken alone, are selected from the group consisting of hydrogen,  $(C_1-C_6)$  alkyl,  $(C_5-C_{14})$  aryl and arylaryl, or when taken together are  $(C_4-C_6)$  alkylidyl or alkyleno, or when individually taken together with  $R^5$  or  $R^7$  is  $(C_2-C_6)$  alkylidyl or alkyleno;

20  $R^7$ , when taken alone, is selected from the group consisting of hydrogen and  $(C_1-C_6)$  alkyl, or when taken together with  $R^6$  or  $R^6'$  is  $(C_2-C_6)$  alkylidyl or alkyleno;

$R^8$ , when taken alone, is selected from the group consisting of hydrogen and  $(C_1-C_6)$  alkyl;

$R^9$  indicates the point of attachment to the *ortho*-carboxyphenyl bottom ring; and

25  $R^{10}$ ,  $R^{11}$ ,  $R^{12}$ ,  $R^{13}$ ,  $R^{14}$ ,  $R^{15}$ ,  $R^{16}$ ,  $R^{17}$ ,  $R^{18}$ ,  $R^{19}$ ,  $R^{20}$  and  $R^{21}$  are each independently selected from the group consisting of hydrogen and  $(C_1-C_6)$  alkyl, or

when  $R^{10}$ ,  $R^{11}$ ,  $R^{12}$  and  $R^{13}$  taken together are  $(C_5-C_{14})$  aryleno or  $(C_5-C_{14})$  aryleno substituted with one or more of the same or different  $(C_1-C_6)$  alkyl, or

when  $R^{18}$ ,  $R^{19}$ ,  $R^{20}$  and  $R^{21}$  taken together are  $(C_5-C_{14})$  aryleno or aryleno substituted with one or more of the same or different  $(C_1-C_6)$  alkyl.

5

6. The rhodamine dye of Claim 5 wherein  $R^2$ , when taken together with  $R^3$  or  $R^3'$  is  $(C_2-C_6)$  alkylidiyl or alkyleno.

7. The rhodamine dye of Claim 6 wherein:

10

alkyl is methanyl, ethanyl or propanyl;

aryl is phenyl or naphthyl;

arylaryl is biphenyl;

alkylidiyl or alkyleno bridges formed by taking  $R^2$  together with  $R^3$  or  $R^3'$ ,  $R^7$  together with  $R^6$  or  $R^6'$ , or  $R^4$  together with  $R^3$  or  $R^3'$ , are ethano, propano, 1,1-dimethylethano,

15

1,1-dimethylpropano and 1,1,3-trimethylpropano;

arylno bridges formed by taking  $R^1$  together with  $R^2$  are benzo or naphtho;

alkylidiyl or alkyleno bridge formed by taking  $R^3$  together with  $R^3'$ , or  $R^6$  together with  $R^6'$ , is butano;

20

alkylidiyl or alkyleno bridges formed by taking  $R^5$  together with  $R^6$  or  $R^6'$  are ethano, propano, 1,1-dimethylethano, 1,1-dimethylpropano and 1,1,3-trimethylpropano; and

arylno bridge formed by taking  $R^{10}$ ,  $R^{11}$ ,  $R^{12}$  and  $R^{13}$  together, or  $R^{18}$ ,  $R^{19}$ ,  $R^{20}$  and  $R^{21}$  together, is benzo.

8. The rhodamine dye of Claim 6 in which L is a bond.

25

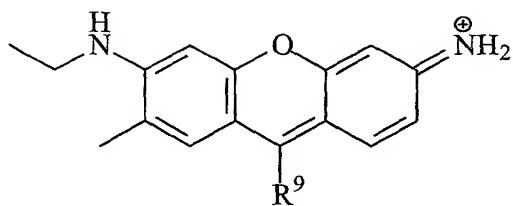
9. The rhodamine dye of Claim 4 in which  $R_x$  is selected from the group consisting of carboxyl, carboxylate, ester and activated ester.

10. The rhodamine dye of Claim 4 in which  $Z^1$  is selected from the group consisting of  $(C_1-C_{12})$  alkylno,  $(C_1-C_{12})$  alkano,  $(C_5-C_{10})$  arylidiyl and heteroarylidiyl, phenylidiyl, phena-1,4-diyl, naphthadiyl, naphtha-2,6-diyl, pyridindiyl and purindiyl.

11. The rhodamine dye of Claim 4 in which Y is selected from the group consisting of:

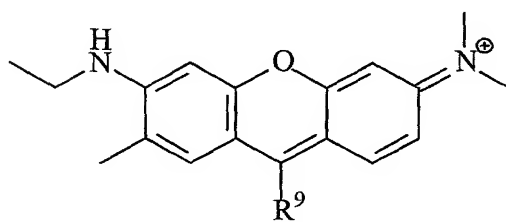
5

(Y-20a)



;

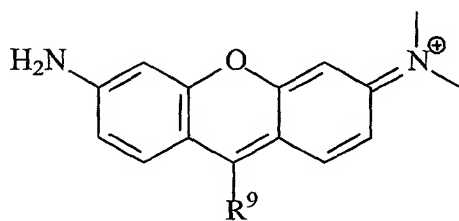
(Y-21a)



;

10

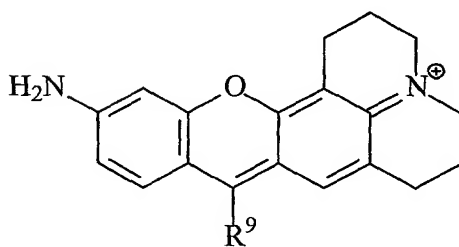
(Y-22a)



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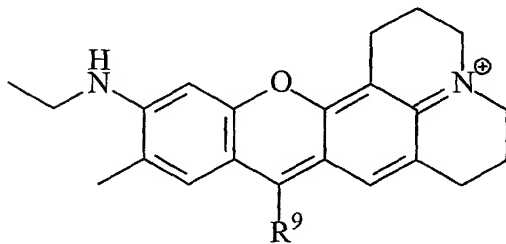
15

(Y-23a)



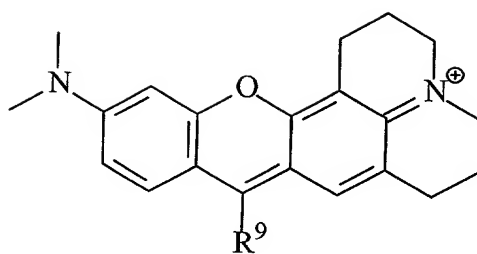
;

(Y-24a)



;

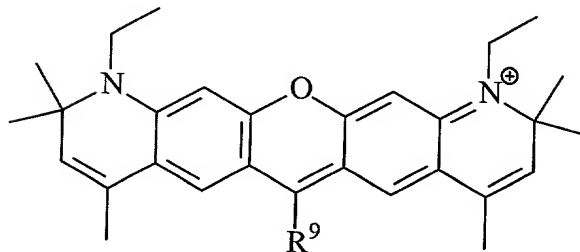
(Y-25a)



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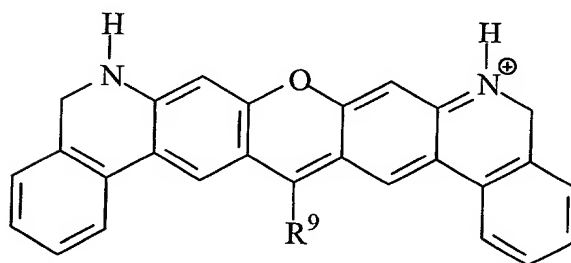
5

(Y-31a)



;

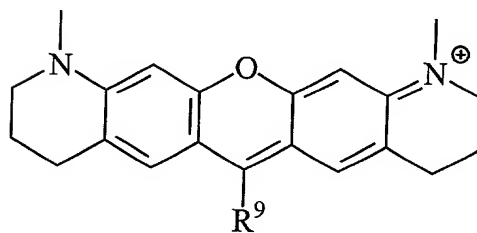
(Y-34a)



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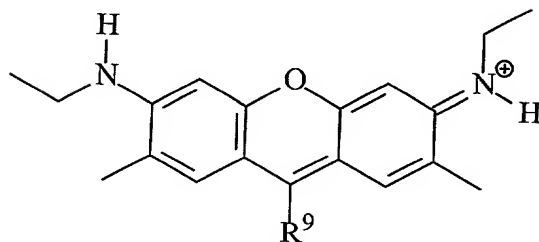
10

(Y-35a)



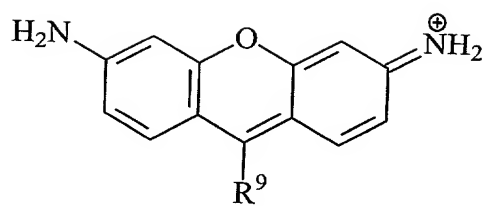
;

15 (Y-36a)



;

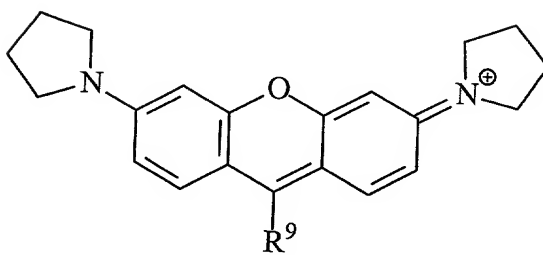
(Y-39a)



;

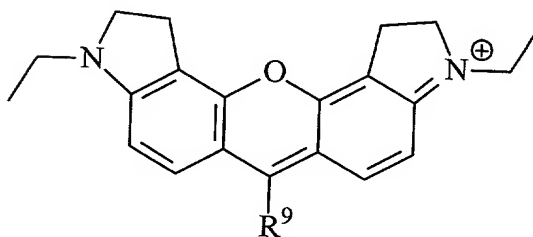
5

(Y-41a)



;

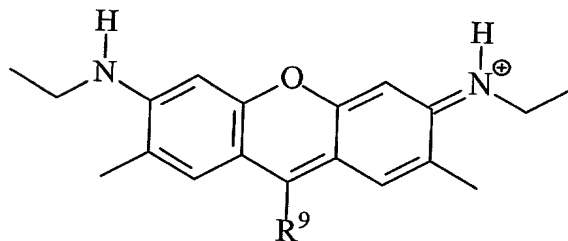
(Y-42a)



;

10

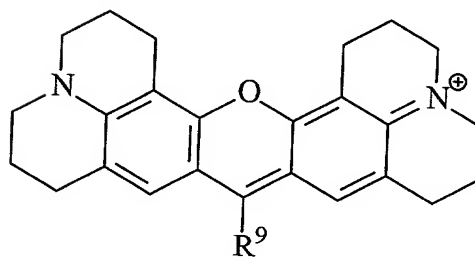
(Y-43a)



;

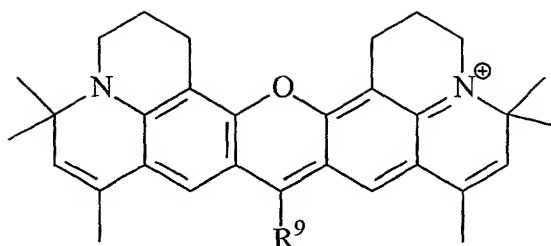
15

(Y-44a)



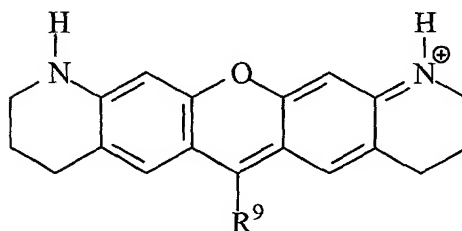
;

(Y-45a)



; and

(Y-46a)



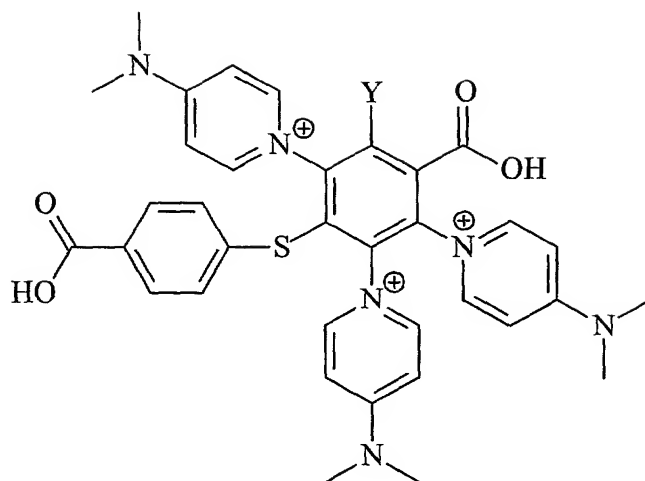
12. The rhodamine dye of Claim 4 in which L is a bond.

13. The rhodamine dye of Claim 4 in which  $R_x$  is selected from the group consisting of carboxyl, carboxylate, ester and activated ester.

14. The rhodamine dye of Claim 4 in which  $Z^1$  is selected from the group consisting of  $(C_1-C_{12})$  alkyleno,  $(C_1-C_{12})$  alkano,  $(C_5-C_{10})$  aryl diyl and heteroaryl diyl, phenyl diyl, phena-1,4-diyl, naphthadiyl, naphtha-2,6-diyl, pyridindiyl and purindiyl.

15. The rhodamine dye of Claim 4 which comprises the structure:





or a salt thereof.

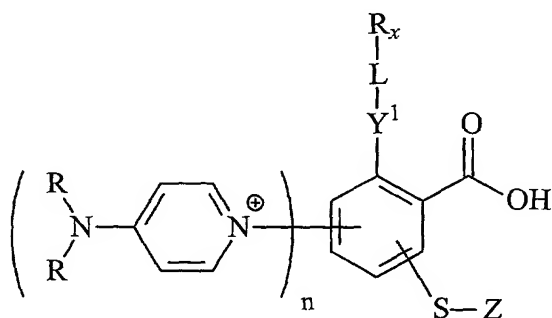
5

16. The rhodamine dye of Claim 15 in which Y is selected from the group consisting of Y-1, Y-2, Y-3 and Y-4.

17. The rhodamine dye of Claim 15 in which Y is selected from the group consisting of Y-20a, Y-21a, Y-22a, Y-23a, Y-24a, Y-25a, Y-31a, Y-34a, Y-35a, Y-36a, Y-39a, Y-41a, Y-42a, Y-43a, Y-44a, Y-45a and Y-46a.

18. The rhodamine dye of Claim 2 which has the structure:

15



wherein:

Y<sup>1</sup> is a rhodamine-type parent xanthene ring attached to the illustrated phenyl group at the xanthene C9 carbon;

L is a bond or linker attached to a xanthene nitrogen atom or a xanthene C4 carbon;

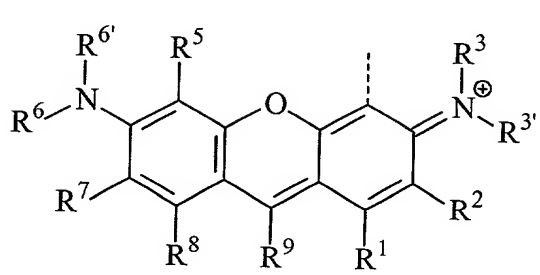
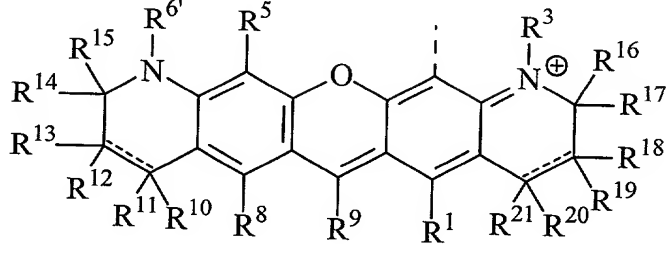
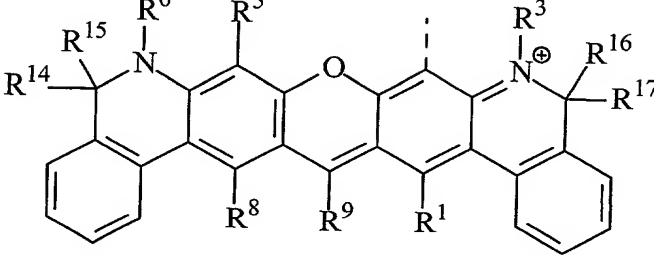
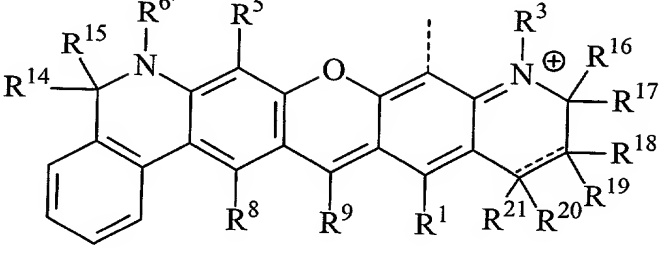
n is 1, 2, or 3; and

R<sub>x</sub> is a reactive functional group.

5

19. The rhodamine dye of Claim 18 in which Y<sup>1</sup> is selected from the group consisting of:

10	(Y-1b)	
15	(Y-2b)	
20	(Y-3b)	
	(Y-4b)	

(Y-1c)	
5 (Y-2c)	
(Y-3c)	
10 (Y-4c)	

wherein the dashed line at the nitrogen or C4 atom indicates the point of attachment of substituent L.

20. The rhodamine dye of Claim 19 wherein:
- alkyl is methanyl, ethanyl or propanyl;
  - aryl is phenyl or naphthyl;
  - aryllaryl is biphenyl;
  - alkyldiyl or alkylene bridges formed by taking R<sup>2</sup> together with R<sup>3</sup>, R<sup>4</sup> together

with R<sup>3</sup>, R<sup>5</sup> together with R<sup>6</sup>, or R<sup>7</sup> together with R<sup>6</sup>, are ethano, propano, 1,1-dimethylethano, 1,1-dimethylpropano and 1,1,3-trimethylpropano;

aryleno bridges formed by taking R<sup>10</sup>, R<sup>11</sup>, R<sup>12</sup> and R<sup>13</sup> together or R<sup>18</sup>, R<sup>19</sup>, R<sup>20</sup> and R<sup>21</sup> together are benzo.

5

21. The rhodamine dye of Claim 18 in which L is selected from the group consisting of (C<sub>1</sub>-C<sub>6</sub>) alkylidiyl, (C<sub>1</sub>-C<sub>6</sub>) alkano, (C<sub>5</sub>-C<sub>20</sub>) arylidiyl, phenylidiyl, phena-1,4-diyl, naphthylidiyl, naphtha-2,6-diyl, naphtha-2,7-diyl, (C<sub>6</sub>-C<sub>26</sub>) arylalkylidiyl -(CH<sub>2</sub>)<sub>i</sub>- $\phi$ - and -(CH<sub>2</sub>)<sub>i</sub>- $\psi$ -, where each *i* is independently an integer from 1 to 6,  $\phi$  is (C<sub>5</sub>-C<sub>20</sub>) arylidiyl, phenylidiyl or phena-1,4-diyl and  $\psi$  is

10 naphthylidiyl, naphtha-2,6-diyl or naphtha-2,7-diyl.

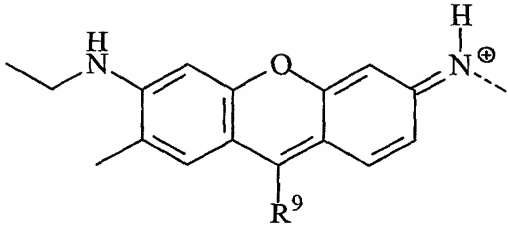
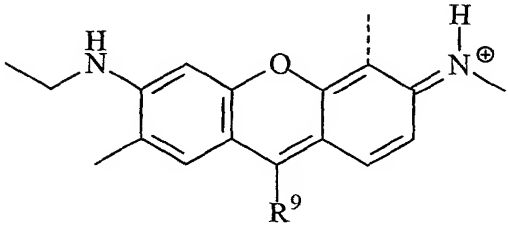
22. The rhodamine dye of Claim 18 in which R<sub>x</sub> is selected from the group consisting of carboxyl, carboxylate, ester and activated ester.

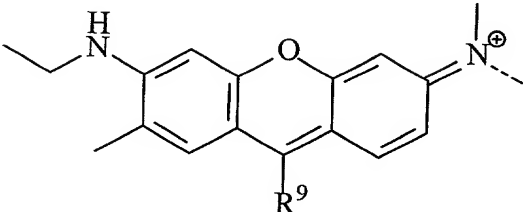
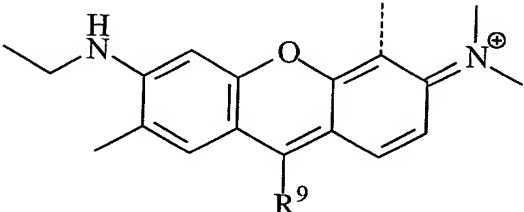
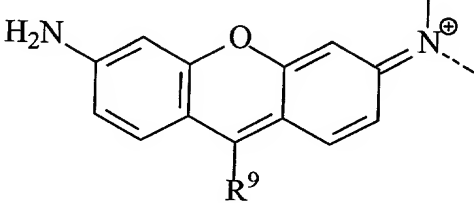
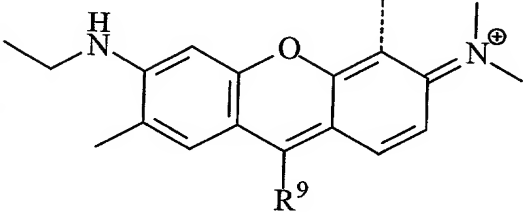
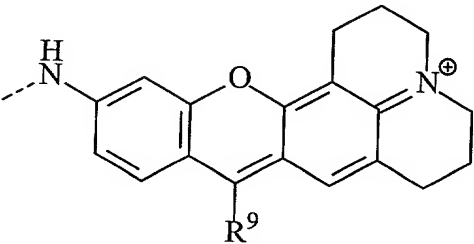
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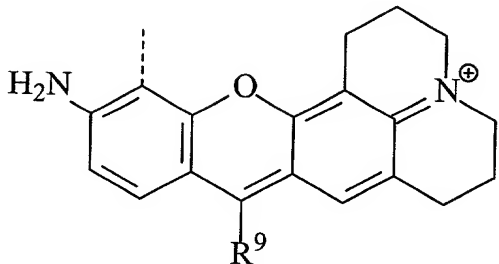
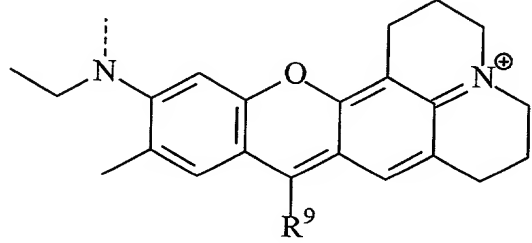
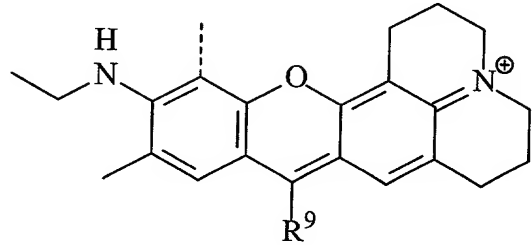
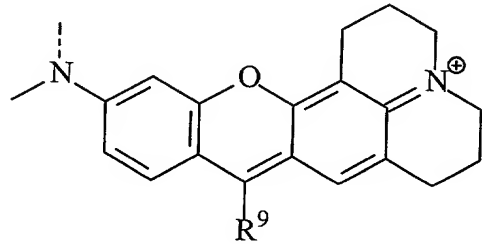
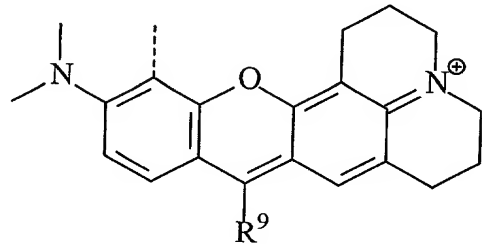
23. The rhodamine dye of Claim 18 in which Z is selected from the group consisting of (C<sub>1</sub>-C<sub>12</sub>) alkyl, (C<sub>1</sub>-C<sub>12</sub>) alkanyl, (C<sub>5</sub>-C<sub>10</sub>) aryl and heteroaryl, phenyl, naphthyl, naphth-1-yl, naphth-2-yl, pyridyl and purinyl.

24. The rhodamine dye of Claim 18 in which Y<sup>1</sup> is selected from the group consisting

20 of:

(Y-20b)	
25 (Y-20c)	

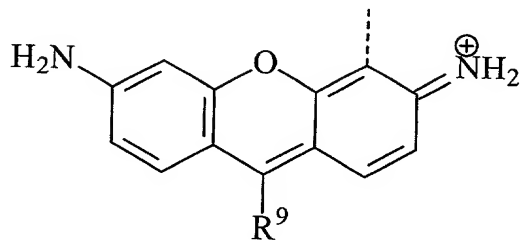
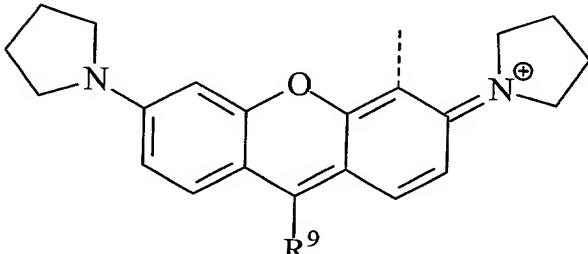
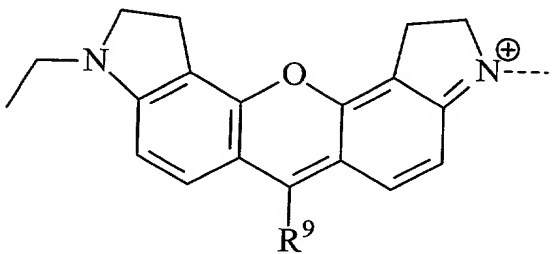
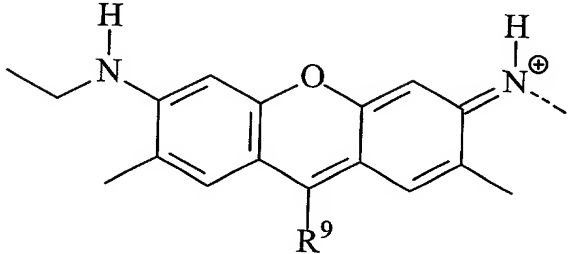
(Y-21b)	
5 (Y-21c)	
(Y-22b)	
10 (Y-22c)	
15 (Y-23b)	

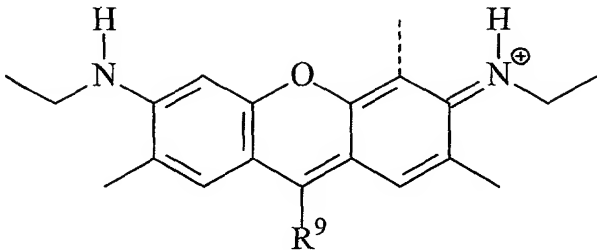
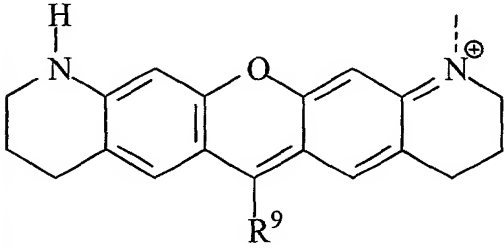
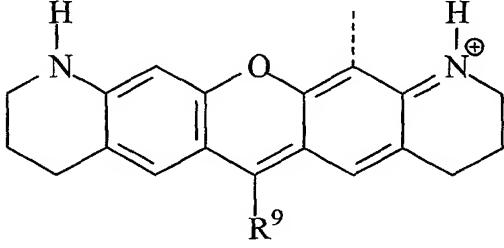
	(Y-23c)	
5	(Y-24b)	
	(Y-24c)	
10	(Y-25b)	
15	(Y-25c)	

15

[illegible]

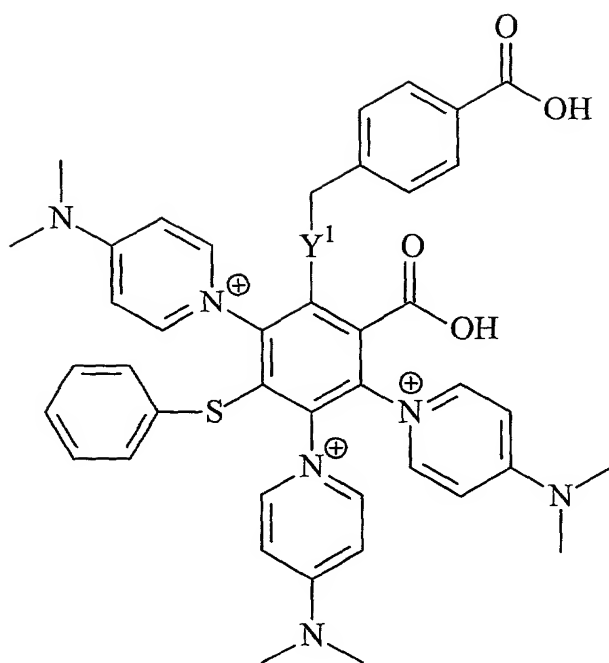


(Y-39c)	
5 (Y-41c)	
(Y-42b)	
10 (Y-43b)	

(Y-43c)	
5 (Y-46b)	
(Y-46c)	

wherein  $R^9$  and the dash at the nitrogen or C4 atom indicates the point of attachment of L.

25. The rhodamine dye of Claim 18 which has the structure:



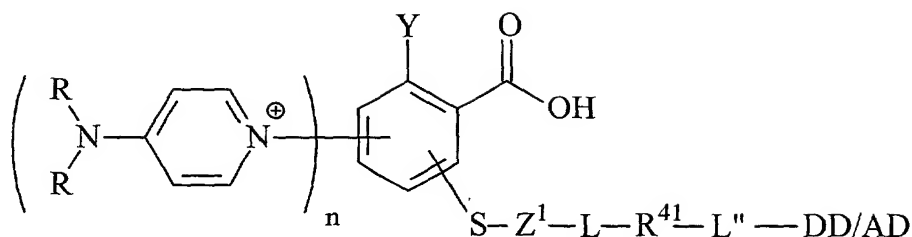
or a salt thereof.

26. The rhodamine dye of Claim 25 in which  $Y^1$  is selected from the group consisting of Y-1b, Y-2b, Y-3b, Y-4b, Y-1c, Y-2c, Y-3c and Y-4c.

27. The rhodamine dye of Claim 25 in which  $Y^1$  is selected from the group consisting of Y-20b, Y-20c, Y-21b, Y-21c, Y-22b, Y-22c, Y-23b, Y-23c, Y-24b, Y-24c, Y-25b, Y-25c, Y-31b, Y-31c, Y-34b, Y-34c, Y-35b, Y-35c, Y-36b, Y-36c, Y-37b, Y-39b, Y-39c, Y-41c, Y-42b, Y-43b, Y-43c, Y-46b and Y-46c.

28. An energy-transfer dye pair comprising a donor dye linked to an acceptor dye, wherein the donor dye or the acceptor dye is a compound according to Claim 1 and either or both of said donor and acceptor dyes include an optional linking moiety.

29. The dye pair of Claim 28 which has the structure:



or a salt thereof, wherein:

5

R<sup>41</sup> is a covalent linkage formed upon reaction between a nucleophile and an electrophile;

L'' is a bond or a linker;

n is 1, 2, or 3; and

DD/AD is a donor dye or an acceptor dye which includes a linking moiety.

10

30. The dye pair of Claim 29 in which Y is selected from the group consisting of Y-1, Y-2, Y-3, Y-4, Y-20a, Y-21a, Y-22a, Y-23a, Y-24a, Y-25a, Y-31a, Y-34a, Y-35a, Y-36a, Y-39a, Y-41a, Y-42a, Y-43a, Y-44a, Y-45a and Y-46a.

15

31. The dye pair of Claim 29 in which L is a bond.

32. The dye pair of Claim 29 in which R<sup>41</sup> has the formula -C(O)NR<sup>45</sup>-, where R<sup>45</sup> is hydrogen or (C<sub>1</sub>-C<sub>6</sub>) alkyl.

20

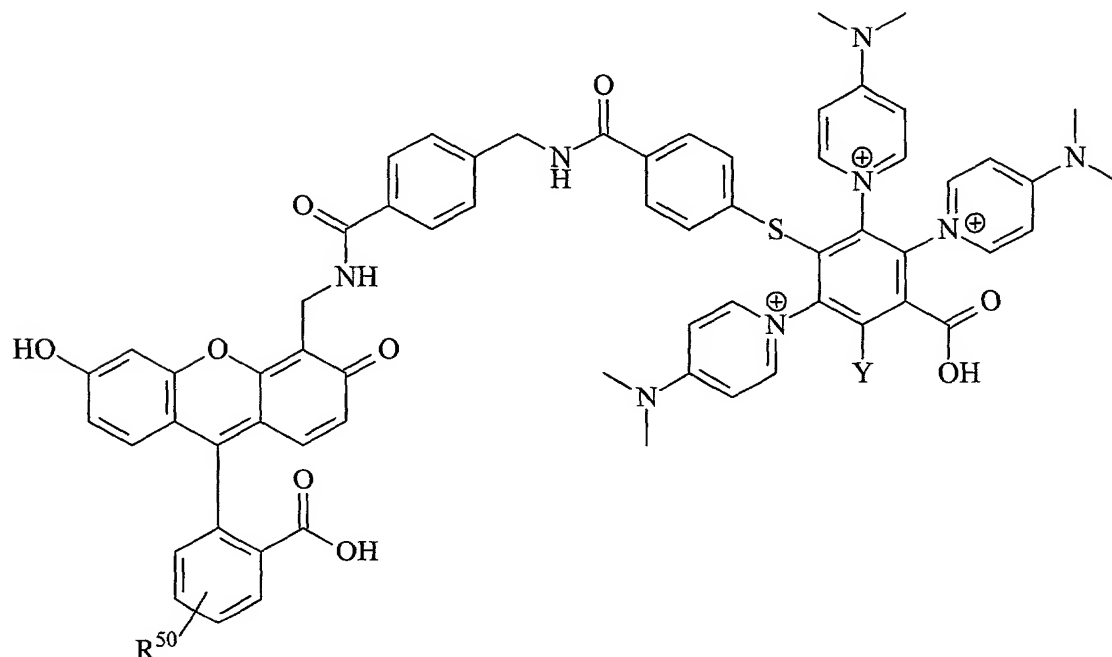
33. The dye pair of Claim 29 in which Z<sup>1</sup> is selected from the group consisting of (C<sub>1</sub>-C<sub>12</sub>) alkylene, (C<sub>1</sub>-C<sub>12</sub>) alkano, (C<sub>5</sub>-C<sub>10</sub>) aryldiyl and heteroaryldiyl, phenyldiyl, phena-1,4-diyl, naphthadiyl, naphtha-2,6-diyl, pyridindiyl and purindiyl.

25

34. The dye pair of Claim 29 in which L'' is -R<sup>43</sup>-Z<sup>3</sup>-C(O)-R<sup>44</sup>-R<sup>45</sup>-, wherein R<sup>43</sup> is (C<sub>1</sub>-C<sub>6</sub>) alkylidiyl, preferably (C<sub>1</sub>-C<sub>3</sub>) alkano, and is bonded to R<sup>42</sup>, where R<sup>42</sup> is O, S or NH; Z<sup>3</sup> is 5-6 membered cyclic alkenyldiyl and heteroalkenyldiyl, (C<sub>5</sub>-C<sub>14</sub>) aryldiyl and heteroaryldiyl; R<sup>44</sup> is O, S or NH; and R<sup>45</sup> is (C<sub>1</sub>-C<sub>6</sub>) alkylidiyl, preferably (C<sub>1</sub>-C<sub>3</sub>) alkano.

35. The dye pair of Claim 29 in which DD/AD is a fluorescein dye in which the linking moiety is a reactive functional group and wherein L" is attached to the fluorescein dye at the xanthene C4 carbon.

5 36. The dye pair of Claim 29 which has the structure:

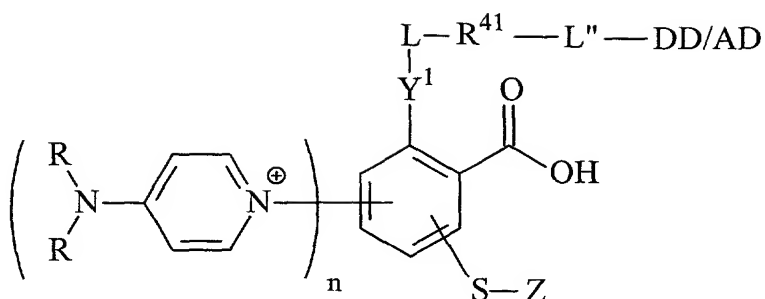


10

wherein, R<sup>50</sup> is a carboxyl, a salt, ester or activated ester thereof.

37. The dye pair of Claim 36 in which Y is selected from the group consisting of Y-1, Y-2, Y-3, Y-4, Y-20a, Y-21a, Y-22a, Y-23a, Y-24a, Y-25a, Y-31a, Y-34a, Y-35a, Y-36a, Y-39a,  
15 Y-41a, Y-42a, Y-43a, Y-44a, Y-45a and Y-46a.

38. The dye pair of Claim 28 which has the structure:



wherein:

5  $R^{41}$  is a covalent linkage formed upon reaction between a nucleophile and an electrophile;

$L''$  is a bond or a linker;

$n$  is 1, 2, or 3; and

DD/AD is a donor dye or an acceptor dye which includes a linking moiety.

10 39. The dye pair of Claim 38 in which  $Y^1$  is selected from the group consisting of Y-1b, Y-2b, Y-3b, Y-4b, Y-1c, Y-2c, Y-3c, Y-4c, Y-20b, Y-20c, Y-21b, Y-21c, Y-22b, Y-22c, Y-23b, Y-23c, Y-24b, Y-24c, Y-25b, Y-25c, Y-31b, Y-31c, Y-34b, Y-34c, Y-35b, Y-35c, Y-36b, Y-36c, Y-37b, Y-39b, Y-39c, Y-41c, Y-42b, Y-43b, Y-43c, Y-46b and Y-46c.

15 40. The dye pair of Claim 38 in which  $L$  is  $(C_1-C_6)$  alkylidiyl or  $(C_1-C_3)$  alkano.

41. The dye pair of Claim 38 in which  $R^{41}$  is an amide of the formula  $-C(O)NR^{45}-$ , where  $R^{45}$  is hydrogen or  $(C_1-C_6)$  alkyl.

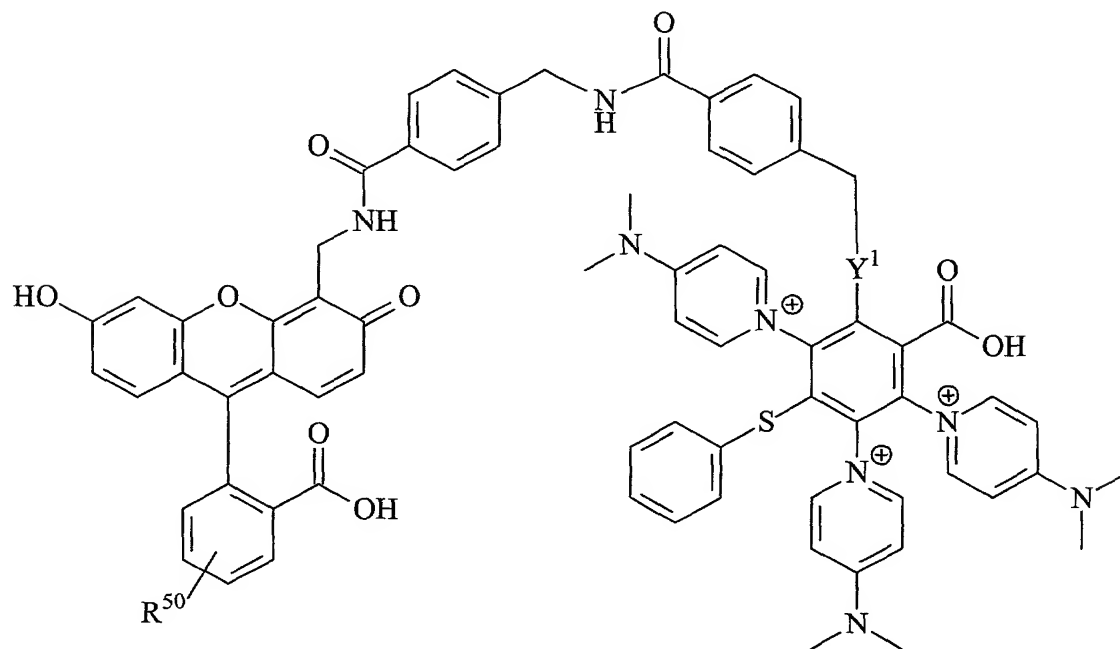
20 42. The dye pair of Claim 38 in which  $Z$  is selected from the group consisting of  $(C_1-C_{12})$  alkyl,  $(C_1-C_{12})$  alkanyl,  $(C_5-C_{10})$  aryl and heteroaryl, phenyl, naphthyl, naphth-1-yl, naphth-2-yl, pyridyl and purinyl.

25 43. The dye pair of Claim 38 in which  $L''$  is  $-R^{43}-Z^3-C(O)-R^{44}-R^{45}-$ , wherein  $R^{43}$  is  $(C_1-C_6)$  alkylidiyl, preferably  $(C_1-C_3)$  alkano, and is bonded to  $R^{42}$ , where  $R^{42}$  is O, S or NH;  $Z^3$  is 5-6 membered cyclic alkenyldiyl and heteroalkenyldiyl,  $(C_5-C_{14})$  aryldiyl and heteroaryldiyl;  $R^{44}$  is

O, S or NH; and  $R^{45}$  is  $(C_1-C_6)$  alkylidyl, preferably  $(C_1-C_3)$  alkano.

44. The dye pair of Claim 38 in which DD/AD is a fluorescein dye in which the linking moiety is a reactive group  $R_x$  and wherein  $L''$  is attached to the fluorescein dye at the 5 xanthene C5 carbon.

45. The dye pair of Claim 38 which has the structure:



10 wherein:

$Y^1$  is selected from the group consisting of Y-20b, Y-20c, Y-21b, Y-21c, Y-22b, Y-22c, Y-23b, Y-23c, Y-24b, Y-24c, Y-25b, Y-25c, Y-31b, Y-31c, Y-34b, Y-34c, Y-35b, Y-35c, Y-36b, Y-36c, Y-37b, Y-39b, Y-39c, Y-41c, Y-42b, Y-43b, Y-43c, Y-46b and Y-46c; and

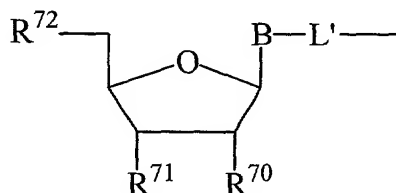
$R^{50}$  is a carboxyl, a salt, ester or activated ester thereof.

15

46. The dye pair of Claim 45 in which  $Y^1$  is selected from the group consisting of Y-1b, Y-2b, Y-3b, Y-4b, Y-1c, Y-2c, Y-3c, Y-4c, Y-20b, Y-20c, Y-21b, Y-21c, Y-22b, Y-22c, Y-23b, Y-23c, Y-24b, Y-24c, Y-25b, Y-25c, Y-31b, Y-31c, Y-34b, Y-34c, Y-35b, Y-35c, Y-36b, Y-36c, Y-37b, Y-39b, Y-39c, Y-41c, Y-42b, Y-43b, Y-43c, Y-46b and Y-46c.

47. A labeled nucleoside/tide or nucleoside/tide analog comprising the rhodamine dye of Claim 2 where Z has the form  $Z^1-L-R^{46}-L'-NUC$ , wherein:

$R^{46}$  is a linkage formed by reaction between an electrophile and a nucleophile; and  $-L'-NUC$  taken together has the structure:



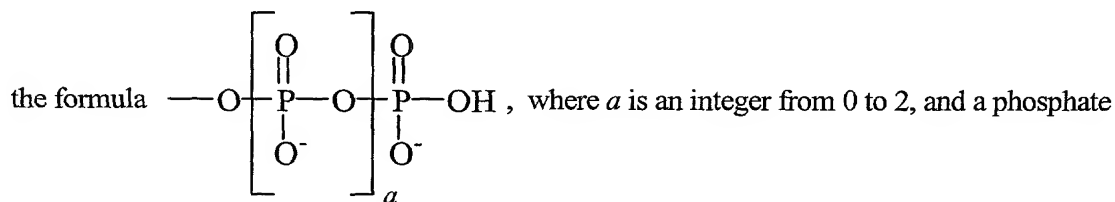
wherein:

B is a nucleobase;

L' is  $(C_1-C_{20})$  alkylidyl and heteroalkylidyl,  $(C_1-C_{20})$  alkylene and heteroalkylene,  $(C_2-C_{20})$  alkyno and heteroalkyno, or  $(C_2-C_{20})$  alkeno and heteroalkeno;

$R_{70}$  and  $R_{71}$ , when taken alone, are each independently selected from the group consisting of hydrogen, hydroxyl and a moiety which blocks polymerase-mediated template-directed polymerization, or when taken together form a bond such that the illustrated sugar is 2',3'-didehydroribose; and

$R_{72}$  is selected from the group consisting of hydroxyl, a phosphate ester having



ester analog, or a salt thereof.

48. The labeled nucleoside/tide or nucleoside/tide analog of Claim 47 where Z has the form  $Z^1-L-R^{41}-L''-DD/AD-L^3-R^{46}-L'-NUC$ , or a salt thereof, wherein:



$R^{41}$  is a covalent linkage formed upon reaction between a nucleophile and an electrophile;

$L''$  is a bond or a linker;

DD/AD is a donor dye or an acceptor dye which includes a linking moiety; and.

5  $L^3$  is a bond or a linker.

49. The labeled nucleoside/tide or nucleoside/tide analog of Claim 47 where Y has the form  $Y^1-R^{41}-L''-DD/AD-L^3-R^{46}-L'-NUC$ , or a salt thereof wherein:

$Y^1$  is Y-1, Y-2, Y-3, or Y-4;

10  $R^{41}$  is a covalent linkage formed upon reaction between a nucleophile and an electrophile;

$L''$  is a bond or a linker;

DD/AD is a donor dye or an acceptor dye which includes a linking moiety; and.

15  $L^3$  is a bond or a linker.

50. A labeled nucleoside/tide or nucleoside/tide analog of Claim 47 where Y has the form  $Y^1-R^{41}-L''-DD/AD$  and Z has the form  $Z^1-L-R^{46}-L'-NUC$ , or a salt thereof; wherein:

$Y^1$  is Y-1, Y-2, Y-3, or Y-4;

20  $R^{41}$  is a covalent linkage formed upon reaction between a nucleophile and an electrophile;

$L''$  is a bond or a linker;

DD/AD is a donor dye or an acceptor dye which includes a linking moiety; and

$Z^1$  is  $(C_1-C_{12})$  alkylidyl,  $(C_1-C_{12})$  alkylidyl independently substituted with one or more of the same or different  $W^1$  groups,  $(C_5-C_{14})$  arylidyl, and  $(C_5-C_{14})$  arylidyl, heteroarylidyl and  
25 heteroarylidyl independently substituted with one or more of the same or different  $W^2$  groups.

51. The labeled nucleoside/tide or nucleoside/tide analog of Claim 47, 48, 49 or 50 which is enzymatically incorporatable.

30 52. The labeled nucleoside/tide or nucleoside/tide analog of Claim 47, 48, 49 or 50 which is a terminator.

53. The labeled nucleoside/tide or nucleoside/tide analog of Claim 47, 48, 49 or 50 which is enzymatically extendable.

54. The labeled nucleoside/tide or nucleoside/tide analog of Claim 47 in which L' is  
5 selected from the group consisting of:

propargyl, where the terminal *sp* carbon is covalently attached to nucleobase B and the terminal methylene (*sp*<sup>3</sup>) carbon is covalently attached to F<sub>x</sub>; and

10  $-C\equiv C-CH_2-O-CH_2-CH_2-NR^{47}-R^{48}-$ , where R<sup>47</sup> is hydrogen or (C<sub>1</sub>-C<sub>6</sub>) alkyl and R<sup>48</sup> is  $-C(O)-(CH_2)_r-$ ,  $-C(O)-CHR^{49}-$ ,  $-C(O)-C\equiv C-CH_2-$  or  $-C(O)-\phi-(CH_2)_r-$ , where each *r* is independently an integer from 1 to 5 and  $\phi$  is C<sub>6</sub> arylidyl or heteroarylidyl and R<sup>49</sup> is hydrogen, (C<sub>1</sub>-C<sub>6</sub>) alkyl or a side chain of an encoding or non-encoding amino acid, and where the terminal *sp* carbon is covalently attached to nucleobase B and the other terminal group is covalently attached to F<sub>x</sub>.

15 55. The labeled nucleoside/tide or nucleoside/tide analog of Claim 48 or Claim 49 in which L<sup>3</sup> is a bond, R<sup>46</sup> the formula  $-C(O)-NHR^{51}$ , where R<sup>51</sup> is hydrogen or (C<sub>1</sub>-C<sub>6</sub>) alkyl.

56. The labeled nucleoside/tide or nucleoside/tide analog of Claim 47 in which nucleobase B is a purine, a 7-deazapurine, an 8-aza,7-deazapurine, a pyrimidine, a normal  
20 nucleobase or a common analog of a normal nucleobase.

57. The labeled nucleoside/tide or nucleoside/tide analog of Claim 47 or Claim 48 in which Y is selected from the group consisting of Y-1, Y-2, Y-3 and Y-4.

25 58. The labeled nucleoside/tide or nucleoside/tide analog of Claim 47 or Claim 48 in which Y is selected from the group consisting of Y-20a, Y-21a, Y-22a, Y-23a, Y-24a, Y-25a, Y-31a, Y-34a, Y-35a, Y-36a, Y-39a, Y-41a, Y-42a, Y-43a, Y-44a, Y-45a and Y-46a.

59. The labeled nucleoside/tide or nucleoside/tide analog of Claim 49 or Claim 50 in  
30 which Y<sup>1</sup> is selected from the group consisting of Y-1b, Y-2b, Y-3b, Y-4b, Y-1c, Y-2c, Y-3c and Y-4c.

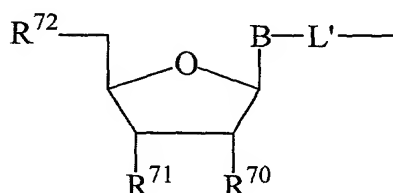
60. The labeled nucleoside/tide or nucleoside/tide analog of Claim 49 or Claim 50 in which Y<sup>1</sup> is selected from the group consisting of Y-20b, Y-20c, Y-21b, Y-21c, Y-22b, Y-22c, Y-23b, Y-23c, Y-24b, Y-24c, Y-25b, Y-25c, Y-31b, Y-31c, Y-34b, Y-34c, Y-35b, Y-35c, Y-36b, Y-36c, Y-37b, Y-39b, Y-39c, Y-41c, Y-42b, Y-43b, Y-46b and Y-46c.

5

61. A polynucleotide labeled with a rhodamine dye according to Claim 1 or an energy-transfer dye pair according to Claim 28.

62. A method of generating a labeled primer extension product, comprising the step of enzymatically extending a primer-target hybrid in the presence of a mixture of enzymatically-extendable nucleotides capable of supporting continuous primer extension and a terminator, wherein said primer or said terminator is labeled with a rhodamine dye according to Claim 1 or an energy-transfer dye pair according to Claim 28.

63. The method of Claim 62 in which the terminator has the structure:



wherein R<sub>70</sub> and R<sub>71</sub>, when taken alone, are each independently selected from the group consisting of hydrogen, halide, and any moiety which blocks polymerase-mediated template-directed polymerization.

64. The method of Claim 62 in which the terminator is a mixture of four different terminators, one which terminates at a template A, one which terminates at a template G, one which terminates at a template C and one which terminates at a template T or U.

65. The method of Claim 62 in which each of the four different terminators is labeled with a different, spectrally-resolvable fluorophore.

66. A labelled rhodamine dye-polypeptide conjugate comprising the rhodamine dye of Claim 1 and a polypeptide, wherein the polypeptide is selected from the group consisting of a peptide, a protein, and an antibody.

5

67. A method of detecting a rhodamine dye-antibody conjugate, in which said conjugate is a rhodamine dye-antibody conjugate according to Claim 66, comprising the steps of:

- (a) binding the conjugate to a peptide or protein, and
- (b) detecting the rhodamine dye-antibody conjugate bound to the peptide or protein.

10

68. The method of Claim 67 in which the conjugate is bound to the peptide or protein in the presence of a second antibody specific for binding said peptide or protein.

69. The method of Claim 68 in which the second antibody is bound to a solid bead or  
15 particle.